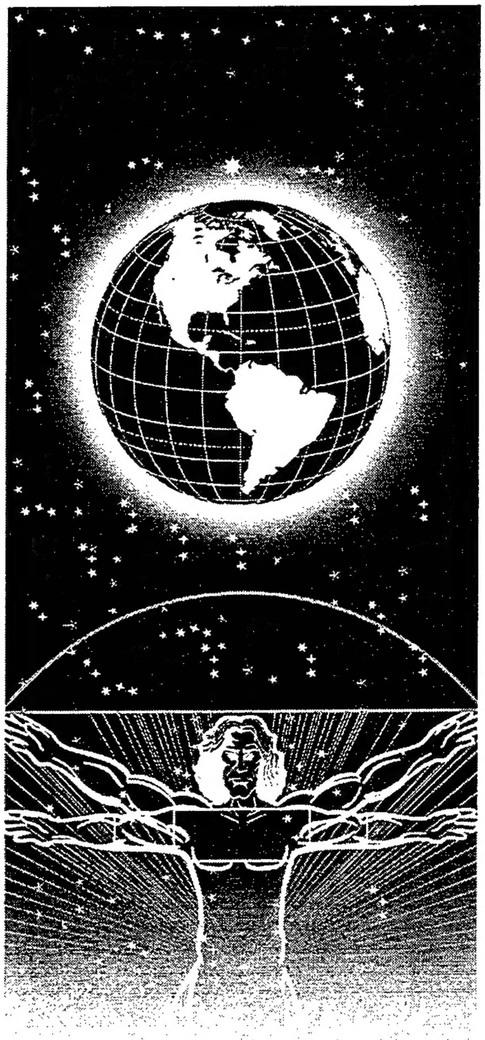
AL/OE-TR-1997-0054



UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

Acute Oral Toxicity Evaluation and Genotoxicity Testing of Hexakis (2,2,2-Trifluoroethoxy) Cyclotriphosphazene, A Replacement Candidate for Ozone Depleting Substances

Robin E. Wolfe
David H. Ellis
Harry F. Leahy
Allen Vinegar

MANTECH - GEO-CENTERS JOINT VENTURE
TOXIC HAZARDS RESEARCH
P. O. BOX 31009
DAYTON OH 45437-0009

S. Sharma

MANTECH ENVIRONMENTAL TECHNOLOGY, INC.
P. O. BOX 12313
RESEARCH TRIANGLE PARK NC 27709

May 1997

Approved for public release;
distribution is unlimited.

Occupational and Environmental Health Directorate
Toxicology Division
2856 G Street
Wright-Patterson AFB OH 45433-7400

NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Armstrong Laboratory. Additional copies may be purchased from:

NATIONAL TECHNICAL INFORMATION SERVICE
5285 PORT ROYAL ROAD
SPRINGFIELD, VIRGINIA 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

DEFENSE TECHNICAL INFORMATION CENTER
8725 JOHN J. KINGMAN RD STE 0944
FT BELVOIR VA 22060-6218

DISCLAIMER

This Technical Report is published as received and has not been edited by the Technical Editing Staff of the Armstrong Laboratory.

TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1997-0054

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Terry A. Childress

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

REPORT DOCUMENTATION PAGE	Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE May 1997	3. REPORT TYPE AND DATES COVERED Final - September-November 1996

4. TITLE AND SUBTITLE Acute Oral Toxicity Evaluation and Genotoxicity Testing of Hexakis (2,2,2-Trifluoroethoxy) Cyclotriphosphazene, A Replacement Candidate for Ozone Depleting Substances	5. FUNDING NUMBERS Contract F41624-96-C-9010 PE 62202F PR 7757 TA 7757A1 WU 7757A102
6. AUTHOR(S) R.E. Wolfe, D.H. Ellis, H.F. Leahy, A. Vinegar, and S. Sharma	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) ManTech - GEO-CENTERS Joint Venture Toxic Hazards Research P.O. Box 31009 Dayton OH 45437-0009	8. PERFORMING ORGANIZATION REPORT NUMBER 4300-9405
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Armstrong Laboratory, Occupational and Environmental Health Directorate Toxicology Division, Human Systems Center Air Force Materiel Command Wright-Patterson AFB OH 45433-7400	10. SPONSORING/MONITORING AGENCY REPORT NUMBER AL/OE-TR-1997-0054
11. SUPPLEMENTARY NOTES	
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.	12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The DoD requires the development of toxicity profiles for chemical substitute candidates, that have little or no ozone depleting potential, to replace ozone depleting substances such as chloro- and fluorocarbons and halons. Hexakis (2,2,2-trifluoroethoxy) cyclotriphosphazene (Hexakis) was identified as a possible replacement candidate for ozone-depleting fire extinguishants. An acute oral toxicity assessment utilizing male and female Fischer 344 rats and genotoxicity testing utilizing the Ames assay were performed on Hexakis. No deaths or signs of toxic stress were observed in any of the rats orally dosed at the limit test value of 5g Hexakis/hg body weight. Body weights during the subsequent 14-day observation period appeared unaffected by treatment. In the Ames assay, Hexakis did not produce mutagenicity in the bacterial (*Salmonella*) system. In summary, Hexakis did not produce toxicity via the oral route of administration and was determined not to be genotoxic.

14. SUBJECT TERMS Hexakis(2,2,2-trifluoroethoxy)cyclotriphosphazene, ozone depleting substance replacement candidate, acute toxicity, limit test, oral gavage, Fischer 344 rats, genotoxicity, Ames Test, Salmonella	15. NUMBER OF PAGES 30 16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

THIS PAGE INTENTIONALLY LEFT BLANK

TABLE OF CONTENTS

LIST OF TABLES

PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxicology Division, Armstrong Laboratory (AL/OET), Wright-Patterson Air Force Base, Ohio, under the ManTech/GEO-CENTERS Joint Venture Toxic Hazards Research contract. This document serves as a final report on the acute oral toxicity evaluation and genotoxicity testing of Hexakis(2,2,2-trifluoroethoxy) cyclotriphosphazene, a replacement candidate for ozone depleting substances. The research described in this report began in September 1996 and was completed in November 1996 under Department of the Air Force Contract No. F41624-96-C-9010. Lt Col Terry A. Childress served as the Contracting Officer's Representative for the U.S. Air Force, Armstrong Laboratory. Darol E. Dodd, Ph.D., served as Program Manager for ManTech/ GEO-CENTERS Joint Venture.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended. The authors gratefully acknowledge the technical assistance of Richard J. Godfrey, Jerry W. Nicholson, Margaret A. Parish, and Darol E. Dodd, Ph.D.

ABBREVIATIONS

°C	Degrees Centigrade
CFCs	Chlorofluorocarbons
DMSO	Dimethyl sulfoxide
DoD	Department of Defense
F-344	Fisher 344 rat(s)
g	Gram(s)
G	Gauge
h	Hour(s)
his^-	Histadine dependent
his^+	Histadine independent
Hexakis	Hexakis(2,2,2-trifluoroethoxy)cyclotriphosphazene
kg	Kilogram
mg	Milligram
mL	Milliliter
mmHg	Millimeters of mercury
SA	Sodium azide
SD	Standard deviation
UV	Ultraviolet
µg	Microgram
2-AF	2-Aminofluorene
9-AA	9-Aminoacridine

SECTION I

INTRODUCTION

Fire extinguishant agents, refrigerants, and other solvents presently in the Department of Defense (DoD) inventory contain halogenated fluorocarbons. Chloro- and bromofluorocarbons (halons) are substances thought to cause ozone depletion in the stratosphere. Environmental concern over this ozone depletion by activity of chlorine radicals from chlorofluorocarbons (CFCs) has led to an international treaty called the Montreal Protocol (1987) which calls for the phaseout of select CFCs and halons by the year 2000. The potential utility of a number of chemical substitutes that have little or no ozone depleting potential are being investigated to meet the demand for alternatives to CFCs and halons.

The DoD requires the development of a toxicity profile for the potential chemical replacements, which includes the results of acute toxicity and genotoxicity testing. Because these replacements are currently being developed and are not manufactured commercially, very little, if any, toxicity information is available in the literature. To initiate responsible industrial hygiene practice within the production area and provide or recommend appropriate protective equipment in the workplace, it is necessary that the operations personnel are aware of the acute health hazards of this compound.

Hexakis(2,2,2-trifluoroethoxy)cyclotriphosphazene (Hexakis) is one of the chemical replacement candidates for ozone depleting substances. Hexakis is a solid material with a vapor pressure of 2 mmHg at 70 °C. Tests performed within this laboratory indicated the vapor pressure of Hexakis was less than 0.1 mmHg at room temperature (20 °C). Therefore, an acute oral toxicity limit test was performed instead of an acute inhalation limit test for this material. The data obtained from this oral toxicity test would provide a measure of toxic potency that can be compared with other chemicals, including other CFCs and halon replacement candidates.

This study was performed to determine the acute toxicity associated with exposure to Hexakis, which was developed by the University of New Mexico as a candidate fire extinguishant. Additionally, Hexakis was examined for its potential to produce genetic toxicity using the *Salmonella*/microsome mutagenesis assay (Ames assay). The species and sex of animals selected for the acute toxicity test were in conformance with the requirements of the U.S. Environmental Protection Agency (1982).

SECTION II

MATERIALS AND METHODS

Test Material

The Hexakis(2,2,2-trifluoroethoxy)cyclotriphosphazene was synthesized and provided by the University of New Mexico, New Mexico Engineering Research Institute. Pertinent chemical and physical properties are listed below.

Hexakis(2,2,2-trifluoroethoxy)cyclotriphosphazene

Boiling Pt.:	248 °C @ 743 mmHg
Vapor Pressure:	2 mmHg @ 70 °C
Melting Pt.:	49 °C
Appearance:	white crystalline solid
Solubility in Water:	negligible

No compositional analysis of Hexakis was performed by this laboratory.

Test Animals

Fischer 344 (F-344) rats (CDF®[F-344]CrlBR), 7 weeks of age, were purchased from Charles River Breeding Laboratory, Wilmington, MA. All animals were identified by tattoo and subjected to a two-week acclimation period. Rats were group housed (two per cage, separated by sex) in clear plastic cages with wood-chip bedding (Sani-Chip®, P.J. Murphy Forest Products, Montville, NJ). Water and feed (Certified Rodent Diet #5002, PMI Feeds, Inc., St Louis,

MO) were available *ad libitum*, except for 12 h prior to oral dosing. Animal room temperatures were maintained at 21 to 25 °C and the light/dark cycle was set at 12-h intervals.

Experimental Design

Oral Toxicity Limit Test

Ten male and ten female F-344 rats were fasted 12 h prior to the administration of the oral gavage dose. Each rat was weighed prior to oral gavage dosing. Five male and five female rats received a test dose of 5 g Hexakis/kg body weight. A dose of 5 g/kg is the limit test value for oral toxicity testing (U.S. EPA, 1982). The Hexakis dosing solution was prepared by suspending the Hexakis in a 1% agar solution (in deionized water) at a concentration of 0.5 g/mL. A 14G oral gavage needle was utilized to deliver the Hexakis suspension. Five male and five female vehicle control animals received 1% agar solution at a dose of 1 mL/100 g body weight. Body weights of surviving rats were measured 1, 2, 4, 7, and 14 days postdosing. Animals were observed daily during the postexposure period, and any clinical signs of toxicity were recorded. Rats were euthanized and gross pathology performed 14 days postexposure. No further testing of Hexakis was performed since no compound related mortality was observed at the limit test dose of 5 g/kg.

SECTION III

RESULTS

Oral Toxicity

Five male and five female rats were orally dosed with 5 g Hexakis/kg body weight. No deaths resulted from the oral administration of the test agent or vehicle, and no signs of toxicity were observed. All rats showed normal weight gains during the 14-day observation period (Tables 1 and 2). No gross lesions were observed at necropsy for any animals on study.

TABLE 1. BODY WEIGHTS[a] OF MALE F-344 RATS AFTER GAVAGE WITH 5 g HEXAKIS/kg BODY WEIGHT

Treatment	Animal Number	Days Posttreatment 0	1	2	4	7	14
Hexakis							
5 g/kg	M-06	245.9	255.6	257.5	254.1	258.5	269.4
5 g/kg	M-07	269.4	269.5	278.5	283.1	286.0	296.4
5 g/kg	M-08	250.5	253.7	254.7	255.2	258.5	267.8
5 g/kg	M-09	268.0	277.0	279.1	282.5	284.6	293.1
5 g/kg	M-10	267.7	271.6	279.1	279.4	283.0	286.3
	Mean	**260.3**	**265.5**	**269.8**	**270.9**	**274.1**	**282.6**
	SD	**11.2**	**10.3**	**12.5**	**14.9**	**14.3**	**13.3**
Control[b]							
1 mL/100 g	M-01	245.2	255.5	254.0	254.8	257.3	262.9
1 mL/100 g	M-02	261.6	269.4	271.2	270.8	273.9	281.8
1 mL/100 g	M-03	236.8	247.4	249.3	248.7	250.0	259.8
1 mL/100 g	M-04	272.5	284.9	286.2	280.4	288.0	291.2
1 mL/100 g	M-05	231.6	236.8	237.5	238.4	240.8	246.6
	Mean	**249.5**	**258.8**	**259.6**	**258.6**	**262.0**	**268.5**
	SD	**17.1**	**18.8**	**19.2**	**16.9**	**18.9**	**17.9**

[a]Weight in grams.
[b]1% agar solution.

TABLE 2. BODY WEIGHTS[a] OF FEMALE F-344 RATS AFTER GAVAGE WITH 5 g HEXAKIS/kg BODY WEIGHT

	Animal	Days Posttreatment					
Treatment	**Number**	**0**	**1**	**2**	**4**	**7**	**14**
Hexakis							
5 g/kg	F-06	153.5	155.4	155.3	151.3	161.2	159.3
5 g/kg	F-07	154.5	162.7	162.0	166.8	169.8	174.4
5 g/kg	F-08	155.5	159.4	158.7	159.6	164.0	170.0
5 g/kg	F-09	164.8	171.3	175.7	175.9	181.5	184.9
5 g/kg	F-10	154.1	161.7	160.1	160.1	166.0	163.7
	Mean	**156.5**	**162.1**	**162.4**	**162.7**	**168.5**	**170.5**
	SD	**4.7**	**5.9**	**7.8**	**9.2**	**7.9**	**9.9**
Control[b]							
1 mL/100 g	F-01	150.1	152.5	153.4	154.6	161.3	162.8
1 mL/100 g	F-02	153.8	160.8	159.9	161.4	167.6	164.8
1 mL/100 g	F-03	157.6	168.0	165.9	171.5	173.8	172.1
1 mL/100 g	F-04	149.8	157.6	160.6	161.2	168.8	166.6
1 mL/100 g	F-05	153.9	157.5	156.4	153.7	153.0	149.3
	Mean	**153.0**	**159.3**	**159.2**	**160.5**	**164.9**	**163.1**
	SD	**3.2**	**5.7**	**4.7**	**7.1**	**8.0**	**8.5**

[a]Weight in grams.
[b]1% agar solution.

SECTION IV

GENOTOXICITY TESTING

Hexakis was examined for its potential to produce genetic toxicity using the *Salmonella*/microsome mutagenesis assay(Ames Test). Results from the Ames Test indicated that Hexakis was not a mutagen for both frame shift (TA98, TA1537) and base-pair substitution (TA100, TA1535) at all the doses tested (0.3125, 0.625, 1.25, 2.5, and 5.0 mg/plate). The number of revertants of all treated groups was the same as in the control (DMSO). The conclusion from this test is that Hexakis is not mutagenic, at least not in the bacterial (*Salmonella*) system.

Background

The *Salmonella*/Mammalian microsome reverse mutation system is a well-defined, short-term assay for the detection of carcinogens/mutagens (Brusick, 1994; Maron and Ames, 1983). It measures the reversion from his$^-$ (histidine dependent) to his$^+$ (histidine independent) induced by chemicals which cause base change or frameshift mutations in the genome of this organism. A reverse mutation can be achieved by base pair changes, which may occur at the site of the original mutation or at a second site in the chromosome, or by frameshift mutations resulted from the addition or deletion of single or multiple base pairs in the DNA molecule.

In this assay, bacteria are exposed to the test agent with and without a metabolic activation system (Aroclor 1254 induced

rat liver S9 with co-factors) and plated onto minimal agar medium which is deficient in histidine. After incubation for 48 h, revertant colonies are counted and compared with the untreated group (DMSO). The mutagenicity of the test agents is assessed from the increased number of revertants.

Methods

Genotype Confirmation

The genotype of each strain was confirmed prior to the mutagenesis study, which included the requirement of histidine (His^-), the sensitivity to crystal violet (rfa mutation) and UV light (uvrB mutation), the resistance to ampicillin (R Factor), and the occurrence of spontaneous revertants.

Mutagenicity Assay - Plate Incorporation

A preliminary range-finding assay was performed using TA100 to determine the test doses of Hexakis. Four tester strains were used in the mutagenicity assay which included TA98, TA100, TA1535, and TA1537 with and without S9 activation.

Preparation of Test Agent

Hexakis was dissolved in DMSO to make a concentration of 5 mg/plate as a stock solution. The stock solutions were then diluted to a dose range of 0.3125-2.5 mg/plate with DMSO. DMSO was used as the untreated (negative) control. Appropriate positive controls were included in each test; 2-aminofluorene (2-AF) with S9 (20 µg/plate) for both TA98 and TA100, sodium azide (SA) without S9 (2 µg/plate) for TA1535, and 9-aminoacridine(9-AA) without S9 (10 µg/plate) for TA1537.

Plate Incorporation Test

The bacterium was cultured in nutrient broth at 37 °C in a gyrorotory incubator for 10-20 h. One-tenth of an mL of the culture was added to 2 mL of top agar. This mixture was melted and kept on a 45 °C heating block, along with 0.1 mL of the test agent, and 0.5 mL of S9 mixture (in $S9^+$ plates only). The contents were mixed and poured onto the surface of a minimal glucose agar plate and spread out evenly. The top agar was allowed to solidify and the plates were incubated at 37 °C for 48 h before the number of revertants per dish was counted by an automatic colony counter. Cultures were set up in triplicate. Only one experiment was performed in this assay.

Results

Genotype Identification

Different genotypes of the tester strains were verified by the standard Ames test procedure of prior to the study. Results confirmed that all the tester strains are qualified for the study.

TABLE 3. RESULTS OF GENOTYPE IDENTIFICATION TESTS

Genotypes	TA98	TA100	TA1535	TA1537
Histidine requirement	+	+	+	+
rfa Mutation	+	+	+	+
uvrB Mutation	+	+	+	+
R factor	+	+	-	-
Spontaneous Revertants	31 ± 1	140 ± 5	25 ± 1	8 ± 1

Dose Selection for Hexakis

Five serial doses of Hexakis were tested using TA100 for dose selection and the results are listed below. The highest dose (5 mg/plate) did not show any toxicity to the tester strain and, therefore, was used as the highest dose. Lower doses were the results of four 2-fold dilutions in the standard assay.

TABLE 4. DOSE SELECTION RESULTS FOR GENOTOXICITY TESTING OF HEXAKIS

Hexakis mg/plate	Revertants/plate (Mean ± SD) $S9^{+}$	Revertants/plate (Mean ± SD) $S9^{-}$
DMSO	149 ± 25	138 ± 8
2-AF	1150 ± 39	151 ± 15
5	115 ± 21	116 ± 14.5
2.5	140 ± 17.9	143 ± 17.3
1.25	117 ± 7.37	134 ± 1.52
0.625	155 ± 11.0	153 ± 34.5
0.3125	110 ± 10.5	133 ± 22.5

Mutagenicity Assay of Hexakis

Results of TA98, TA100, TA1535, TA1537 are summarized in Table 5, where the data is expressed as the average (± SD) revertant number per plate calculated from triplicate plates. There was no dose-dependence or increase in the number of revertants at all five doses when compared with control in all four test strains.

Conclusion

The data from the study indicate that Hexakis is not a mutagen causing either base-pair substitution or frameshift mutation in the bacterial (*Salmonella*/Microsome assay) system.

TABLE 5. MUTAGENICITY ASSAY OF HEXAKIS IN AMES TEST

Treatment	Number of Revertants/Plate[a]							
	TA98		TA100		TA1535		TA1537	
	S9+	S9-	S9+	S9-	S9+	S9-	S9+	S9-
Negative Control DMSO	28 ± 4.6	22 ± 4	113 ± 8.7	120 ± 3.2	29 ± 3	24 ± 1	7 ± 2	5 ± 2
Positive Control 2-AF	1870 ± 75	35 ± 2.3	1174 ± 75	154 ± 8				
SA						551 ± 5.5		
9-AA								12 ± 1.5
Hexakis (mg/plate)								
5.0	36 ± 1.2	33 ± 1.0	131 ± 21	140 ± 11	25 ± 5	23 ± 1.0	7 ± 2.6	5 ± 1.5
2.5	33 ± 1.1	28 ± 1.5	127 ± 7	120 ± 8	23 ± 1	24 ± 1.2	7 ± 1.7	6 ± 1.5
1.25	32 ± 1.5	26 ± 1.5	123 ± 9	138 ± 15	24 ± 2	28 ± 2.0	6 ± 2.0	8 ± 1.0
0.625	30 ± 2.8	26 ± 2.1	131 ± 11	132 ± 7	26 ± 3	25 ± 3.7	6 ± 1.5	4 ± 1.0
0.3125	34 ± 3.7	28 ± 1.0	121 ± 24	141 ± 12	26 ± 2	28 ± 2.6	5 ± 1.7	4 ±<0.1

[a]Mean ± SD.

SECTION V

DISCUSSION

In the oral toxicity evaluation of Hexakis(2,2,2-trifluoroethoxy)cyclotriphosphazene, no deaths or signs of toxic stress were observed in any of the animals dosed at the limit test value of 5 g Hexakis/kg body weight. Body weights during the subsequent 14-day observation periods appeared unaffected by treatment. Under the conditions of the limit test, Hexakis did not produce toxicity via the oral route of administration. When examined for its potential to produce genetic toxicity using the *Salmonella*/microsome mutagenesis assay (Ames assay), Hexakis did not produce mutagenicity in the bacterial (*Salmonella*) system, and was therefore determined not to be genotoxic.

SECTION VI

REFERENCES

Brusick, D. 1994. In: *Principles and Methods of Toxicology,* Third Edition, A.W. Hayes, ed. Raven Press Ltd., NY. p. 563.

Maron, D.M. and B.N. Ames. 1983. Revised methods for the *Salmonella* mutagenicity test. *Mutation Res.* **113**:173-215.

U.S. Environmental Protection Agency. 1982. Health Effects Test Guidelines (Report No. EPA 56016-82-001). Washington, DC: Office of Pesticides and Toxic Substances.

APPENDICES A through E

GENOTOXICITY DATA

Appendix A. Ames Test Range-Finding Experiment

Strain TA100

Experiment Date 9-24-96

Agent	Dose/plate	Revertants/plate Count 1	Count 2	Count 3	Mean	SD
		S9+				
DMSO		177	144	127	149	25
2-AF	20 μg	1195	1132	1123	1150	39
Hexakis (mg)	5.0	136	116	93	115	21
	2.5	164	120	127	140	17.9
	1.25	112	115	126	117	7.3
	0.625	168	150	148	155	11
	0.3125	100	109	121	110	10.5
		S9-				
DMSO		143	143	129	138	8.1
2-AF	20 μg	138	149	168	151	15.2
Hexakis (mg)	5.0	115	103	132	117	14.5
	2.5	147	124	158	143	17.3
	1.25	134	133	136	134	1.5
	0.625	152	189	120	154	34.5
	0.3125	116	159	126	134	22.5

Appendix B. Ames Test

Strain TA98

Experiment Date 10-08-96

		Revertants/plate				
Agent	Dose/plate	Count 1	Count 2	Count 3	Mean	SD
		S9+				
DMSO		31	23	31	28	4.6
2-AF	20 µg	1860	1800	1950	1870	75
Hexakis (mg)	5.0	37	35	35	36	1.2
	2.5	32	34	34	33	1.1
	1.25	34	33	31	32	1.5
	0.625	29	34	29	30	2.8
	0.3125	39	33	32	34	3.7
		S9-				
DMSO		26	18	21	22	4.0
2-AF	20 µg	34	34	38	35	2.3
Hexakis (mg)	5.0	32	34	33	33	1.0
	2.5	30	27	27	28	1.5
	1.25	28	25	26	26	1.5
	0.625	26	29	25	26	2.1
	0.3125	28	29	27	28	1.0

Appendix C. Ames Test

Strain TA100

Experiment Date 10-08-96

		Revertants/plate				
Agent	Dose/plate	Count 1	Count 2	Count 3	Mean	SD
			S9+			
DMSO		106	111	123	113	8.7
2-AF	20 μg	1260	1150	1090	1174	75
Hexakis (mg)	5.0	108	136	149	131	20.9
	2.5	124	123	135	127	6.6
	1.25	125	114	132	123	9.0
	0.625	137	118	138	131	11.2
	0.3125	102	112	148	121	24
			S9-			
DMSO		130	118	112	120	9.2
2-AF	20 μg	154	162	150	154	8.0
Hexakis (mg)	5.0	146	127	147	140	11.2
	2.5	111	126	124	120	8.1
	1.25	123	138	153	138	15
	0.625	125	134	139	132	7.0
	0.3125	129	152	144	141	11.6

Appendix D. Ames Test

Strain TA1535

Experiment Date 10-08-96

		Revertants/plate				
Agent	Dose/plate	Count 1	Count 2	Count 3	Mean	SD
			S9+			
DMSO		27	28	33	29	3.2
Hexakis	5.0	21	23	30	25	4.7
mg	2.5	23	24	22	23	1.2
	1.25	22	23	26	24	2.1
	0.625	29	27	23	26	3.1
	0.3125	24	25	28	26	2.1
			S9-			
DMSO		23	25	24	24	1.1
SA	20 µg	556	545	551	551	5.5
Hexakis	5.0	22	24	23	23	1.2
(mg)	2.5	23	23	25	24	1.2
	1.25	26	28	30	28	2.1
	0.625	30	23	24	25	3.7
	0.3125	30	25	29	28	2.6

Appendix E. Ames Test

Strain TA1537

Experiment Date 9-24-96

		Revertants/plate				
Agent	**Dose/plate**	**Count 1**	**Count 2**	**Count 3**	**Mean**	**SD**
			S9+			
DMSO		8	5	9	7	2.1
Hexakis (mg)	5.0	8	9	4	7	2.6
	2.5	8	8	5	7	1.7
	1.25	7	4	8	6	2.3
	0.625	5	6	8	6	1.5
	0.3125	4	7	4	5	1.7
			S9-			
DMSO		2	4	8	5	2.5
9-AA	10 µg	11	12	14	12	1.5
Hexakis (mg)	5.0	6	3	5	5	1.5
	2.5	5	8	6	6	1.5
	1.25	8	9	7	8	1.2
	0.625	5	4	3	4	1.1
	0.3125	4	4	4	4	0